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Early interaction of feline calicivirus with cells in culture

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Summary. The kinetics and biochemical properties of feline calicivirus (FCV) attachment to Crandell-Reese feline kidney cells were determined. Maximum binding was observed at pH 6.5. Cells in suspension at 4 °C bound virus more efficiently than cells in monolayers at 4 °C or 37 °C. High initial binding rate was observed in monolayers or cells in suspension and proceeded to a maximum at 90 min, although half maximal binding was observed as early as 15 min. Binding was specific and competitively blocked by serotypically homologous or heterologous FCV as well as by San Miguel sea lion virus. Treatment of cells with proteases increased FCV binding, whereas phospholipase had no effect on virus attachment. Conversely, cells treated with neuraminidase followed by O-glycanase treatment showed a decreased binding ability. Cells of feline origin bound FCV very efficiently, and non-permissive cells showed a poor binding ability. Following transfection of viral RNA, infectious virus could be recovered from all non-permissive cells, except from Madin-Darby canine kidney cells. These results suggest that FCV binds to a receptor in which carbohydrates may be an important component and that FCV replication in non-permissive cells is primarily restricted by the absence of appropriate receptors on the cell surface.

Introduction

Caliciviruses are a family of small non-enveloped viruses ranging in size from 35 to 40 nm in diameter [37]. These viruses are characterized by their unique capsid morphology which consist of a single protein of 60 to 70 KiloDaltons (KD) [38]. Caliciviruses possess a positive-sense, non-segmented, polyadenylated RNA genome of approximately 7.69 kb [7] to which a protein (VpG) is covalently attached to the 5′ end [6]. Members of the *Caliciviridae* family include feline calicivirus (FCV), San Miguel sea lion virus (SMSV), vesicular exanthema of swine virus, and rabbit hemorrhagic disease virus [34–36]. Caliciviruses have also been identified in other animal species but are not well characterized [10, 13, 14, 40, 41].

Although the molecular biology of caliciviruses has been investigated, no information is available concerning the early interaction of these viruses with cells in culture. The first event in viral infection involves interaction of the virus with receptors on the cell surface. Viral receptors are thought to be the major determinants of viral replication, tissue tropism and pathogenesis [9, 15–17, 21, 26, 31, 33]. The number and distribution of viral receptors varies greatly according to cell type, and the virus-receptor interaction depends on factors like temperature, pH of the binding media and the presence of divalent cations. Poliovirus receptors, for example, are found only in primate cells [9], and the HIV receptor, the CD4 molecule, is restricted to a few human cells [15]. The interaction of foot-and-mouth disease virus (FMDV) with cell receptor is inhibited at pH values lower than 6.4, and is optimized at 4 °C [5]. In contrast, rhinovirus and Theiler's murine encephalomyelitis virus interaction with receptors is reduced at low temperatures [19, 27]. Moreover, the presence of EDTA in the binding media diminishes binding of FMDV and rhinovirus to cell receptors, but does not interferes with poliovirus binding [5, 27], suggesting that some viruses may require divalent cations for proper binding.

In this study, we investigated the binding characteristics of FCV with feline cells and its interaction with various non-permissive cells in culture.

Materials and methods

Cells and virus

Crandell-Reese feline kidney cells (CRFK) [11] were grown in 490 cm^2 roller bottles on Hank's minimal essential media (MEM) and supplemented with 5% fetal calf serum. Feline calicivirus strain CFI/68 [12], and KCD [18] were obtained from American Type Culture Collection. The FCV strain NADC-1 was isolated from a cat at the Ames, Iowa, animal shelter (W.L.M.). To determine cell type tropism, various cell lines, specified in the figure legends, were obtained from the American Type Culture Collection or the Cytology Group of the National Veterinary Services Laboratory (NVSL, Ames, IA, U.S.A.).

Virus purification

Feline calicivirus strain CFI/68 was propagated by infection of CRFK cells at a multiplicity of infection of 10 PFU per cell. After adsorption for 1 h at 37 °C, the viral inoculum was removed and 10 ml of serum-free media was added and incubation was continued at 37 °C until cytopathic effect (CPE) was complete. To obtain radioactively-labeled virus, CRFK cells were incubated in methionine-cysteine free Hank's MEM without serum. Trans 35-S label (ICN Biomedicals, Inc.) containing radioactive methionine and cysteine was added to a concentration of 30 μCi/ml 4 h after infection. Infected cells were harvested when cells detached freely from the flask surface. The supernatant fluid was frozen and thawed twice to release viruses and the cell debris was removed by centrifugation at 12 000 × **g** for 30 min at 10 °C in a Beckman JA-20 rotor. The supernatant was layered onto a step gradient containing 1 ml of 55% sucrose and 6 ml of 27.5% sucrose, prepared in R-buffer (10 mM Tris-hydrochloride [pH 7.2], 150 mM NaCl, 50 mM $MgCl_2$) [1]. The virus was banded by centrifugation at 100 000 × **g** for 2 h in a swinging bucket rotor. The virus band was collected, dialyzed overnight against R-buffer and layered over a linear density gradient of 1.28 to 1.6 g/ml of CsCl in R-buffer. Isopycnic banding of the virus was achieved by centrifugation at

150 000 × **g** for 18 h in a SW 40 rotor. The viral band was removed from the gradient and dialyzed against R-buffer. For radioactively-labeled virus, the gradient was fractionated from the bottom into 0.5 ml fractions, and 5 µl of each fraction was used for the determination of radioactivity by liquid scintillation counting. A second aliquot of 5 µl was removed from each fraction for slot-blot hybridization of viral RNA [39]. The density of each fraction was determined by refractometry (Fisher Scientific Co.) and infectivity of each fraction was performed by plaque assay on 35 mm petri dishes. Following purification, viruses were examined by electron microscopy.

Virus quantification

The number of PFU/ml in each aliquot from the gradient was determined. Briefly, 100 µl of a tenfold dilution of each fraction was adsorbed on CRFK cells grown in 35 mm petri dishes. After 1 h at 37 °C, the inocula was removed, the cells washed, and 5 ml of 0.5% agarose in serum-free MEM layered on top. Plaques were visualized at 48 h post infection (p.i.) by staining the cells with crystal violet (0.02%). The number of virus particles were determined by absorption at 259 nm in a 1 cm path using $E1\%/259 = 76$ [2].

Virus binding assay

Measurements of virus attachment were performed on monolayer cell culture or cells in suspension by a modification of the procedure described by Baxt et al. [5].

Method 1

To determine binding of virus to monolayers, CRFK cells were grown in 24-well plates to a concentration of approximately 8×10^5 cells/well. After removing the media and washing the cells with serum-free MEM, 10 µl of MEM containing radioactively-labeled virus was added. The multiplicity of infection (particles/cell) used are indicated in the figures and legends. Plates were incubated at 4 °C or 37 °C, and gently rocked every 10 min. At various times p.i. the inoculum was removed and the plates washed with 0.5 ml of cold phosphate buffered saline (PBS). The intact cell monolayer was solubilized with 0.2 ml of PBS containing 1% Triton-X-100 and transferred to scintillation vials in which 1.5 ml of scintillation fluid was added. Radioactivity was determined in a Packard TRI-CARB 2200 CA liquid scintillation analyzer. All experiments were repeated in triplicate, on different days, and equivalent results were consistently obtained.

Method 2

To determine binding of virus to cells in suspension, CRFK cells were washed once with PBS and removed with 5 mM EDTA in PBS without Ca2+ or Mg2+. After detachment, the cells were washed once in MEM 5% calf serum, followed by two washes in cold serum-free MEM in which they were resuspended to a concentration of 5×10^7 cells/ml. Aliquots containing 5×10^5 cells were placed individually on 1.5 ml conical plastic tubes and centrifuged for 5 sec at 12 000 × **g** in a microcentrifuge. The supernatant was carefully removed and the cells resuspended in 20 µl of MEM containing radioactively-labeled virus at concentrations indicated in the figures. Binding was allowed to occur at 4 °C, or as otherwise stated. The reaction was quenched at specific times by the addition of 0.2 ml of cold PBS and centrifugation at 12 000 × **g** for 5 sec. The supernatant fluid was removed and a second wash step was performed. The pellet was collected as described for method 1. Binding of radioactive FCV to other cells was assessed by the same method.

Receptor saturability

To determine the number of cell receptors for FCV, CRFK cells were prepared as described in method 2 and incubated with increasing amounts of radioactively labelled virus. Cell associated radioactivity was determined after incubation at 4 °C for 90 min.

Competition experiments

Cells in suspension were incubated with 20-fold excess of unlabeled homologous (CFI/68) or serologically heterologous (KCD and NADC) FCV. San Miguel sea lion virus [37], a calicivirus from pinnipeds, and the non-enveloped parvovirus feline panleukopenia virus (FPV) [23] were also used in competition experiments. After incubation at 4 °C for 90 min with non-radioactive virus, cells were pelleted, washed, and incubated with radioactively-labeled virus for an additional hour at 4 °C. The amount of cell-associated radioactively was determined as described above.

Transfection of cells

Aliquots containing 5 μg/ml of viral genomic RNA [28] were pelleted and resuspended in 100 μl of OPTI MEM 1 (Gibco-BRL). For transfection, 20 μl of Lipofectin (Gibco-BRL) was diluted to a final volume of 100 μl of OPTI MEM 1 and mixed with the solution containing the viral RNA [30]. The mixture was gently vortexed and incubated on ice for 5–10 min when OPTI MEM 1 was added to a final volume of 1 ml. Confluent monolayers of cells on 24-well plates were washed twice with serum-free MEM and incubated with OPTI MEM 1 for 15 min before transfection. The media was removed and the cells transfected with 20 μl/well of the RNA-Lipofectin preparation. After 3 h at 37 °C, the inocula was replaced by 0.5 ml of serum-free MEM and incubation was allowed to proceed for 24–48 h. The supernatant was collected and viral progeny measured by plaque assay. Lipoinfection using viral particles was also completed using a multiplicity of infection of 20. After 2 h, the inocula was replaced by MEM containing anti FCV neutralizing polyclonal antisera at a dilution of 1:500 and incubated for 30 min. Control viral infections were conducted simultaneously without Lipofectin in each cell line, as shown in Fig. 7. Viral progeny was measured by plaque assay as described.

Enzyme treatment of cells

To determine the nature of the cell receptor for FCV, aliquots of cells in suspension were treated with the enzymes listed in Table 1 at the specified conditions. Cells were then washed twice in MEM containing 2% fetal calf serum and 2 μM of PMSF to remove the enzymes and the remaining binding ability was determined by incubating the cells with radioactively-labeled virus for 60 min at 4 °C. The cell-associated radioactivity in each treatment was measured and compared with untreated cells.

Enzymes, chemicals, and isotopes

Translabeled 35-S methionine/cysteine was purchased from ICN Biomedicals, Inc. The following enzymes were purchased from Boehringer Mannheim Biochemicals: chymotrypsin, pronase, proteinase K, neuraminidase, and papain. Trypsin, α-glycosidase, β-glycosidase, α-amylase, chondroitinase ABC lyase, acylase, and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma. Phospholipase A was obtained from Calbiochem-Behring Corp. O-glycanase was purchased from Genzyme Corp.

Results

Virus purification

Virus preparations used for binding experiments were obtained from lysates of infected cells by two centrifugation steps, through sucrose and CsCl, respectively. A predominant peak of radioactivity was observed in the CsCl fraction with a density of 1.37 g/ml (Fig. 1A), which corresponded to the previously reported density of FCV virion [37]. Higher virus titers were also observed in fractions having a peak of radioactivity and slot-blot results clearly demonstrated a greater amount of viral RNA in these fractions (Fig. 1A). Gel electrophoresis of radioactively-labeled virus showed a single band of radioactivity (data not shown) corresponding to the single capsid protein of FCV. Viruses obtained by this method consisted of uniform particles, with a typical cup-shaped morphology, as demonstrated by electron microscopy (Fig. 1B).

Effect of pH on virus attachment

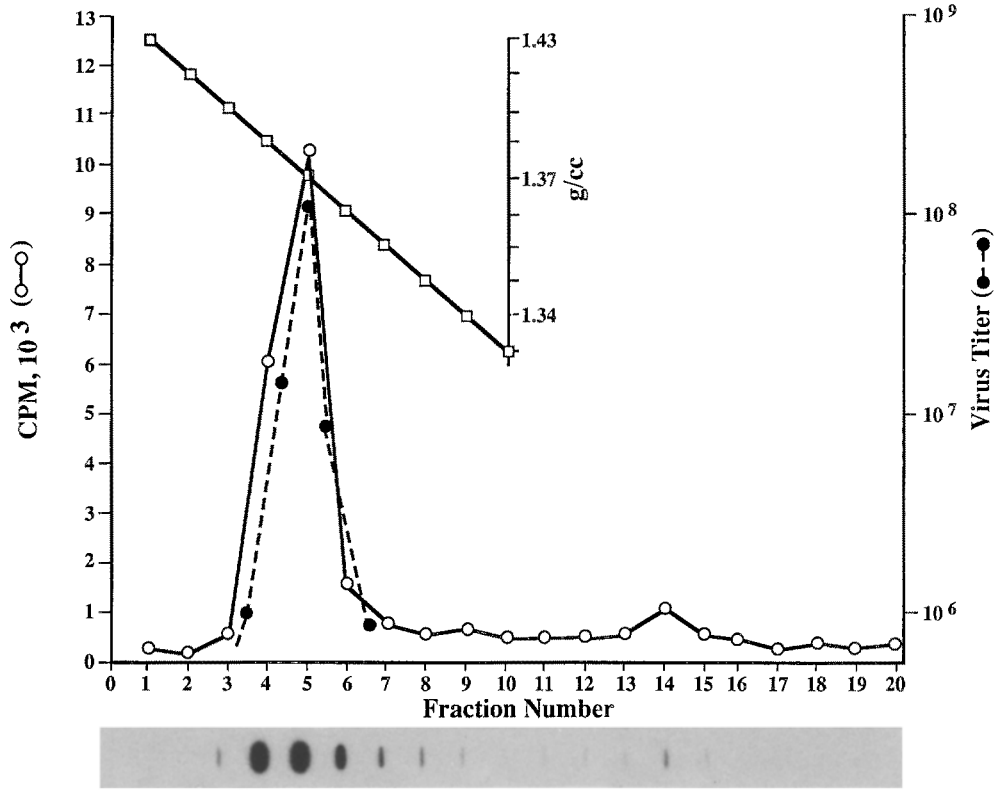
The effect of pH on FCV binding to CRFK cells was determined for a series of values between pH 5.0 and 9.0 (Fig. 2). Maximum binding of FCV was observed at pH 6.5, although there was no large difference in the binding ability of the virus in the pH range between 6.0 and 8.0. Binding occurred to a lesser extent at pH 5.0 to 6.0 and decreased sharply at pH 9.0. The pH was maintained between 6.5 and 7.0 for subsequent virus binding experiments. These results correlated well with those observed with plaque assays in which a maximum number of plaques was consistently observed at pH 6.0 (Fig. 2).

Kinetics of FCV attachment to CRFK cells

Figure 3 illustrates the binding of 35-S methionine labeled FCV strain CFI/68 to CRFK cells. Viruses readily associated with cellular receptors and half maximum of total binding was complete by 15 min. However, binding of FCV to cells in monolayers occurred to a lesser extent than binding to cells in suspension. Adsorption of viruses occurred at a greater initial rate and to a greater extent at 4 °C than at 37 °C. Cells in suspension at 4 °C bound viruses more efficiently than cells in monolayers and a plateau was reached as soon as 30 min after virus addition to CRFK cells.

Saturability of FCV binding

To determine whether FCV binding to CRFK cells was saturable, increasing amounts of radioactively-labeled FCV was incubated with a constant number of CRFK cells in suspension. The number of viral particles which became cell associated increased linearly with viral input (Fig. 4). A plateau was reached at multiplicity of infection of approximately 1.5 to 3×10^3 virus/cell, indicating a finite number of receptors on the cell surface (Fig. 4).



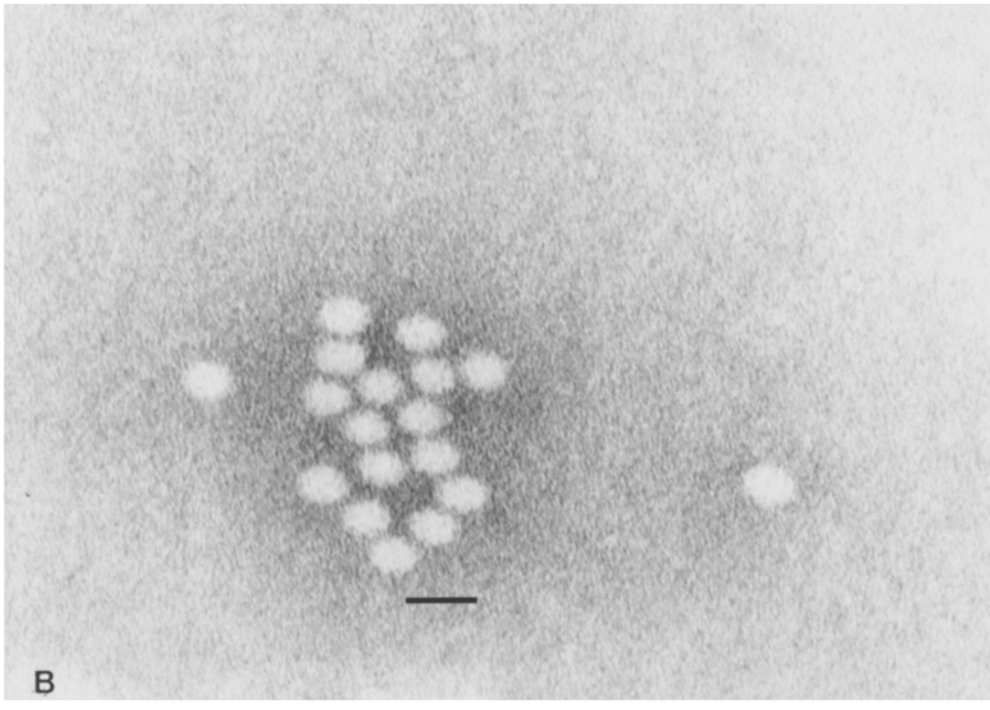


Fig. 1. FCV purification. **A** [35S]-methionine labeled FCV serotype CFI/68 was purified by centrifugation through CsCl linear gradient (1.28 to 1.6g/cc). Fractions of 0.5 ml were collected from the bottom to the top. A portion of each fraction was analyzed by liquid scintillation spectrometry (—○—) or infectivity (—●—). Dot blot analysis of each fraction is depicted below the fraction number. Density of gradient fractions and infectivity were accomplished as described in the text. **B** Electron micrograph of purified FCV. Magnification is at 221 000 ×. Bar: 50 nm

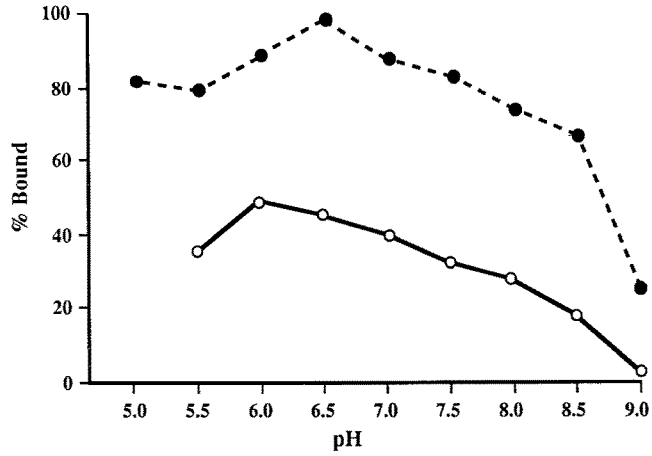


Fig. 2. Effect of pH on FCV binding and plaque formation. Cells in suspension were incubated at the indicated pH at 4 °C with radioactively-labeled virus. After 1 h, cells were washed and the amount of virus bound was determined. For plaque formation, monolayers of CRFK cells were preincubated for 15 min with MEM and further incubated with virus for 1 h at the indicated pH at 37 °C. Petri dishes were overlaid with MEM 0.5% agarose and incubated at 37 °C. Values indicated are the average of two or more experiments. (—○—)% of maximum cpm bound; (—●—) Number of plaques × 10^7

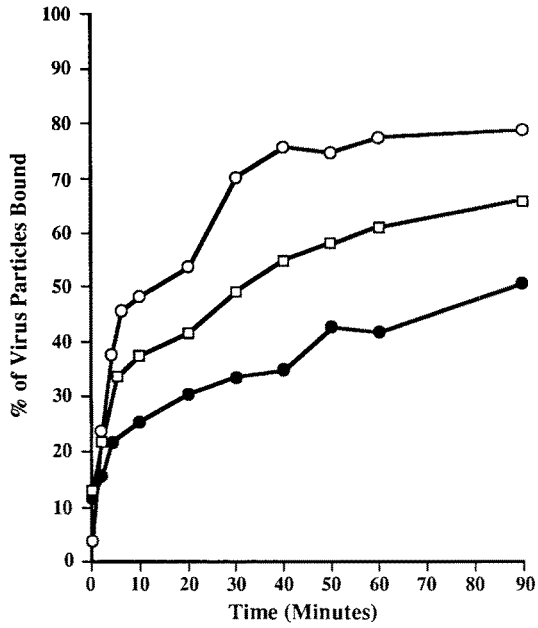


Fig. 3. Kinetics of FCV attachment to CRFK cells. Radioactively-labeled FCV serotype CFI/68 was bound to CRFK cells is suspension at 4 °C (—○—), in monolayers at 4 °C (—□—) or in monolayers at 37 °C (—●—). The reaction was quenched at each time point by washing the cells with cold MEM. The cell-associated radioactivity was measured by liquid scintillation spectrometry and is expressed as a percentage of total input. Results are the average of two or more experiments

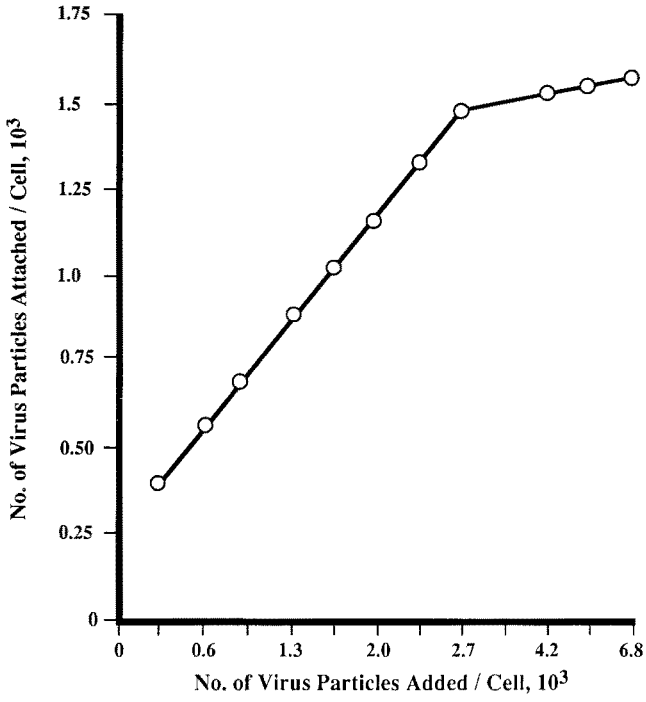


Fig. 4. Number of putative attachment sites for FCV on CRFK cells. An increasing number of radioactively-labeled FCV particles was added to a constant number of cells (5×10^5cells) in suspension. After 90 min at 4 °C, cells were washed twice and the number of bound virus was determined

Specificity of FCV binding

To determine whether FCV attachment was specific and if different isolates of FCV shared the same receptor, the ability of unlabeled FCV isolate CFI/68 to compete with 35-S labeled CFI/68 for attachment to CRFK cells was assessed (Fig. 5). Unlabeled FCV strains KCD and NADC, SMSV, and FPV were also assessed for their ability to block attachment of radioactively-labeled FCV. The attachment of radiolabeled FCV was inhibited by excess of homologous unlabeled virus (Fig. 5). Feline calicivirus isolates KCD and NADC were also able to reduce attachment of CFI/68 to a similar extent as the homologous virus. Interestingly, San Miguel sea lion virus 1 (SMSV 1), a calicivirus from pinnipeds, was also able to prevent binding of CFI/68 to CRFK cells, even though it does not replicate in feline cells. Binding of FCV to CRFK cells was not affected by the presence of FPV.

Fig. 6. Binding of FCV to different cells of feline origin. Cells in suspension (5×10^5 cells) were allowed to react with 1×10^8 virus particles at 4 °C for 60 min. Cells were rinsed and associated radioactivity was determined by liquid scintillation spectrometry. Values indicated are in percentage of total input and represent the average ± SEM of two or more experiments

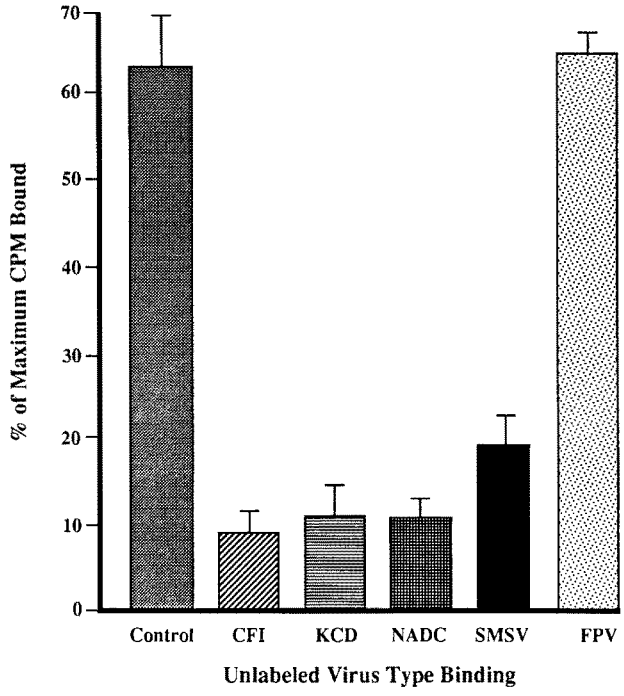
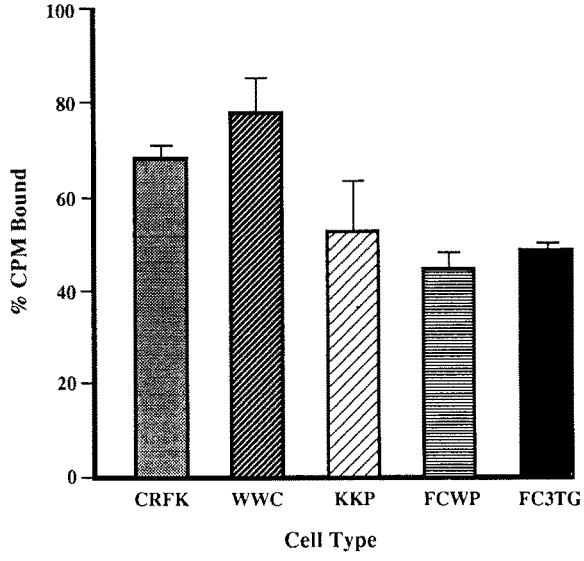


Fig. 5. Competition binding experiments among caliciviruses. CRFK cells in suspension (5×10^5cells) were allowed to react with an excess (1×10^9 particles) of homologous (CFI/68) or heterologous (KCD, NADC) unlabeled virus for 90 min at 4 °C. A related calicivirus from pinnipeds, San Miguel sea lion virus (SMSV), and the non-enveloped feline parvovirus (FPV) were also used in competition experiments. Radioactively-labeled virus was added after washing cells twice with cold MEM, and allowed to react for a further hour at 4 °C. Cell-associated radioactivity was estimated as a percentage of total input and represents the average ±SEM of two or more experiments



FCV cell tropism and replication in non-permissive cells

Cell tropism of FCV was assessed by incubation of radioactively-labeled virus with cells in suspension for 60 min at 4 °C. The amount of bound virus is represented as percentage of virus input. FCV bound readily and efficiently to all feline cell lines tested (Fig. 6). Highest binding was observed with WWC and CRFK cells. Somewhat lower binding was observed with the feline tongue cells Fc3Tg and with the primary cells KKp and FCWP. Binding to the other mammalian cells could not be demonstrated at levels higher than background except MDCK cells which bound FCV at approximately 30% that of CRFK cells (Fig. 7A).

To investigate the role of receptors on FCV replication, the cationic phospholipid Lipofectin was used to transfect a variety of non-permissive cells with viral RNA or viral particles. CRFK cells were used as a control for comparisons of viral progeny, which was measured by plaque assay. Virus yield in the various transfected cell lines were as specified in Fig. 7B. Viral replication was readily

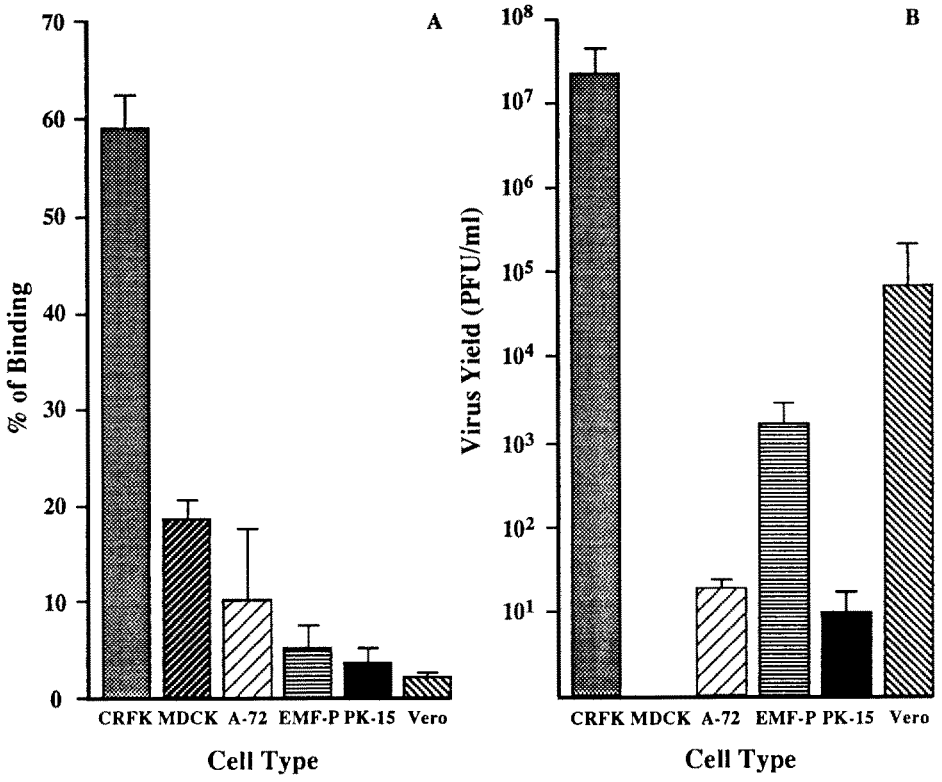


Fig. 7. Feline calicivirus interaction with non-permissive cells. **A** Cells in suspension (5×10^5 cells) were allowed to react with radioactively-labeled FCV CFI/68 at 4 °C for 1 h. Cell-associated radioactivity was determined after washing the cells twice with cold MEM. Results are represented as percentage of total input and are the average ±SEM of two or more experiments. **B** Monolayers of cells in 24-well plates were transfected with viral RNA as described in the text . Virus yield was measured by standard plaque assay procedures on monolayers of CRFK cells grown in 35 mm petri dishes

Table 1. Effect of enzymatic treatment of CRFK cells on FCV binding

Enzyme	Conc.	% Binding compared with control
None		100
Phospholipase A[a]	200 μg/ml	105
Trypsin[b]	50 μg/ml	122
Chymotrypsin[b]	100 μg/ml	118
Proteinase K[b]	50 μg/ml	132
Pronase[b]	25 U/ml	115
Papain[c]	200 μg/ml	136
Neuraminidase[d]	400 mU/ml	101
α-glycosidase[e]	100 μg/ml	95
β-glycosidase[e]	1 mg/ml	92
α-amylase[e]	100 μg/ml	102
O-glycanase[f]	2 mU/ml	69
Chondroitinase ABC lyase[e]	1 U/ml	97
Acylase[e]	100 μg/ml	103

[a] Reaction conditions: pH 7.5, 25 °C, 30 min
[b] Reaction conditions: pH 7.5, 25 °C, 15 min
[c] Reaction conditions: pH 5.5, 25 °C, 15 min
[d] Reaction conditions: pH 5.0, 25 °C, 60 min
[e] Reaction conditions: pH 7.0, 37 °C, 60 min
[f] Cells were treated with 400 mU of neuraminidase per ml at pH 5.0, 25 °C, for 60 min prior to the treatment with O-glycanase at pH 6.8, 37 °C, 2 h. Buffer: [a,b,c,d] serum-free MEM; [e] PBS; [f] 20 mM sodium phosphate

observed in CRFK cells transfected with viral RNA or viral particles. Non-permissive cells transfected with viral RNA showed dispersed, rounded cells that detached from the monolayer, except MDCK cells. Control non-permissive cells infected with virus showed no CPE and virus could not be recovered from the supernatant at 24 h p.i. (data not shown).

Effect of enzymes on FCV binding

To determine the biochemical nature of FCV receptors, CRFK cells in suspension were submitted to a variety of enzymatic treatments. Binding ability of treated cells was assessed and compared with that of untreated cells. Cells, virus, and enzyme concentrations, as well as reaction conditions, were as specified in Table 1. Binding of FCV to CRFK cells was enhanced on cells treated with proteases (trypsin, chymotrypsin, proteinase K, pronase, and papain). Phospholipase A had no effect on virus binding. Glycosidases had little or no effect on FCV binding, except O-glycanase. When CRFK cells were treated with neuraminidase followed by O-glycanase treatment, virus binding ability of the cells was reduced by approximately 30%. Binding was also partially inhibited by sodium periodate treatment of the cells.

Discussion

Receptors on the cell surface are used by viruses to gain entry into cells where they replicate. The virus-receptor interaction is influenced by factors such as temperature, pH and divalent cations. No information regarding these early events of viral replication is available for feline calicivirus. Furthermore, no information on the interaction of caliciviruses with non-permissive cells had been reported.

The binding characteristics of FCV to CRFK cells and its interactions with other mammalian cells were examined in this study. The binding of FCV CFI/68 to CRFK cells occurred independently of the temperature. The overall FCV binding was greater at 4 °C. Similar results have been reported for foot-and-mouth disease (FMDV) [5], encephalomyocarditis virus [32], and mengo virus [29]. Virus binding occurred rapidly and half maximum of total binding was observed as early as 15 minutes. Cells in suspension bound virus at a greater initial rate than cells in monolayers, probably because more receptors on the cell surface are available for interaction with viral proteins. Lower binding observed with cells in monolayers at 37 °C may be accounted for by spontaneous virus elution at the physiological temperature.

The rate of FCV attachment to CRFK cells was highly dependent on virus and cell concentration. When the volume of the binding media was increased, but not the virus or cell concentration, the rate of attachment decreased significantly. The interaction of FCV with cellular receptors was dependent on divalent cations. Increasing concentrations of EDTA greatly reduced the amount of virus binding (data not shown). The requirement of divalent cations for proper interaction between a virus and its cell receptor has been well characterized for human rhinovirus type 2 [25, 27] and foot-and-mouth disease virus [5]. Accordingly, viruses bound at 4 °C could be easily removed from the cell surface by the addition of EDTA. However, when binding was performed at 37 °C, EDTA had little effect on virus elution. These observations indicated that EDTA interferes with virus attachment but has no effect on internalized viruses. Consequently, it was proposed that divalent cations directly contribute to the proper juxtaposition of the ligands by maintaining the virus or the cell receptor in a position that maximizes binding.

Binding of FCV to CRFK cells did not vary significantly from pH 6.0 to 8.0, but was slightly decreased at pH values lower than 6.0. Maximum and minimum binding was observed at pH 6.5 and 9.0, respectively. Similar pH dependence on the interaction of virus and cell receptors were also observed for FMDV [5], rabies virus [43], and simian virus 40 [8]. Furthermore, the ability of FCV to form plaques on a plaque assay was consistently higher on cells held at pH 6.0 during the adsorption period.

It was demonstrated in this study that FCV exhibits specific saturable binding to CRFK cells. The number of receptors was estimated from the relationship between input virus and the amount of radioactively labelled virus that bound to the cells. The binding curve indicated that the number of virus bound per cell increased linearly until 1.5 to 3×10^3 viruses bound to each cell.

The number of receptor observed in this study correlates well with data reported for other virus-cell systems [5, 24, 43]. It has been assumed and extensively reported that viruses from the same group share or compete for the same receptors on permissive cells [5, 19, 42, 43]. Similarly, our experiments have demonstrated that different FCV isolates competed for the same receptors on CRFK cells, demonstrating that binding was specific. Non-specific binding observed in the presence of an excess of unlabeled homologous virus averaged approximately 10%. Surprisingly, SMSV 1 prevented FCV from binding to the cells, even though CRFK cells are non-permissive to SMSV 1. Conversely, FCV did not bind to Vero cells, in which SMSV 1 replicates, suggesting that SMSV 1 may recognize a related receptor on CRFK cells. Reciprocal experiments were not carried out and the binding characteristics of SMSV will be investigated in future studies. Binding of FCV to CRFK cells was not affected by FPV, an unrelated non-enveloped feline virus.

Binding of FCV was observed with all feline cells tested. Differences observed on the amount of virus attached to an individual cell type may reflect the number and distribution of receptor on the cell surface. FCV binding to non-permissive cells occurred to a lesser extent and may be non-specific. However, MDCK cells show a higher binding ability but no attempts were made to assess specificity of binding or virus internalization.

Non-permissive cells may prevent viral replication because the absence of specific receptors. Such inhibition in replication may be overcome by transfecting these cells with viral particles or viral genome. The preformed liposome, Lipofectin, has been used to bypass the membrane lipid bilayer, delivering virus particles [3, 22] or nucleic acids into the cells [30], greatly improving the understanding of the early events of virus replication. To address the question of receptor specificity, FCV particles and FCV RNA were transfected to permissive and non-permissive cells. Transfected RNA could be replicated in non-permissive cells. Cells transfected with viral particles provided similar results (data not shown). However, viral progeny could not be recovered from MDCK-transfected cells, which was the sole non-permissive cell line that bound virus to a larger extent. Such cells probably restricted viral replication at the translation or post-translational level, rather than at the receptor level as observed for the remaining non-permissive cells.

Information on the biochemical nature of receptors is commonly sought by treating cells with enzymes and analyzing the effect of such treatment on virus binding to the cells. CRFK cells treated with a variety of different enzymes were used for binding experiments. The results of these assays suggest that a carbohydrate moiety may be an important component of the receptor. The enzyme O-glycanase, with specifically removes the Gal-β (1, 3) GalNac core disaccharides from serine and threonine residues of glycoproteins, reduced FCV binding to CRFK cells by approximately 30%. Neuraminidase treatment of the cells prior to the addition of O-glycanase is recommended to remove potential inhibitors of O-glycanase such as N-acetyl neuraminic acid, a common substituent of O-linked carbohydrates. Treatment of cells with O-glycanase alone had no effect

on FCV binding. Further evidences for the involvement of carbohydrates on the attachment site for FCV came from studies wherein sodium periodate treated cells had a reduced binding ability (data not shown). Interestingly, a variety of other small non-enveloped viruses have been shown to require O-linked sugar residues for adequate binding [4, 8, 19, 20].

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